

# Biofilms and wound infections.

Biofilms in chronic wounds?

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# The chronic wound

- Arises from different acute wounds that requires different initial treatment



# Bacterial Biofilm

- Bacterial biofilm is still one of the main reasons for unsuccessful treatment of implant related infections
- Biofilm formation is probably present in all chronic infections
- Treatments that takes biofilm into account are more likely to be successful

Wolcott RD, Rhoads DD (2008) A study of biofilm-based wound management in subjects with critical limb ischaemia. J Wound Care 17: 145-2, 154.



# Pressure Ulcers

Cause:

Sustained or repetitive pressure in the immobilized patient



Treatment:

Off-loading mattresses, cushions, ambulation



# Diabetic Foot Ulcers



Cause:

Sustained or repetitive pressure in the neuro-ischemic foot



Treatment:

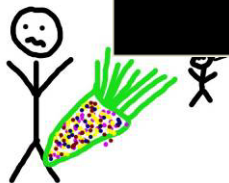
Restoration of circulation. Off-loading in therapeutic footwear, cast, orthoses. Surgery





# Controversies

- A diabetic foot ulcer:





# Venous Leg Ulcers

Cause:

High hydrostatic pressure in veins in lower extremity due to venous valvular defects

Treatment:

Compression bandage or stockings





# The Chronic Wound

- A wound that heals slower than expected?
- A wound that has not healed within 3 months?
- A wound that will never heal?
- A wound that will not heal despite effective treatment of cause?



# Causes of the chronic wound (not complete)

- Immunological defects in the patient
  - Cancer
  - HIV infection
  - Inborn defects
  - Diabetes
  - Immunosuppression
  - Malnutrition
- Defects in wound healing
  - Diabetes
  - Inborn defects
  - Malnutrition
- Infection



# Infection definitions

- Contaminated
  - No bacteriological proliferation
- Colonized wound
  - Bacterial proliferation but no inflammation
- Infection
  - More than  $10^5$  bacteria per gram tissue?
  - Clinical infection (inflammation)?



# Venous Leg Ulcer Bacteriology

- Gødsbøl et al, Copenhagen Wound Healing Center:
  - *Staphylococcus aureus* (93.5%)
  - *Enterococcus faecalis* (71.7%)
  - *Pseudomonas aeruginosa* (52.2%)
  - Coagulase-negative staphylococci (45.7%)
  - *Proteus* species (41.3%)
  - Anaerobic bacteria (39.1%)



# Venous Leg Ulcer Bacteriology II

- Other works:
  - *Staphylococcus aureus* 38-88%
  - *Pseudomonas aeruginosa* 26-51%
- Differences probably due to sampling technique and clinical population





# Chronic wound characteristics

- Slow fibroblasts
- Elevated Metalloproteases (MMPs)
- Decrease in Tissue Inhibiting Metalloprotease (TIMP)
- High rate of Polymorph Nuclear Neutrophils (PMN) in the chronic wound



# Role of PMN in wound healing

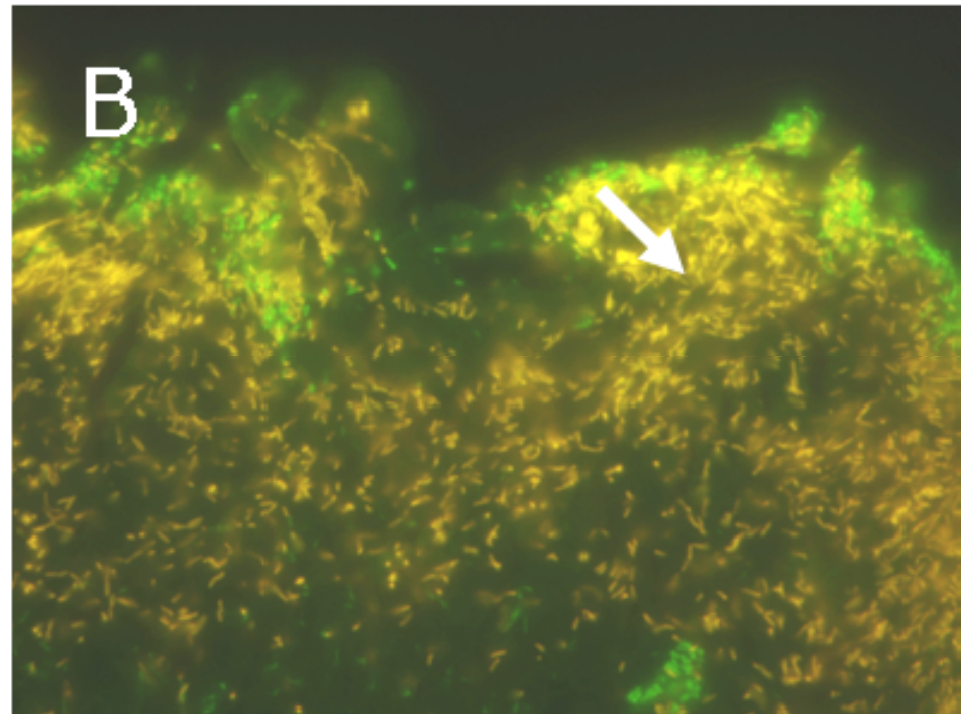
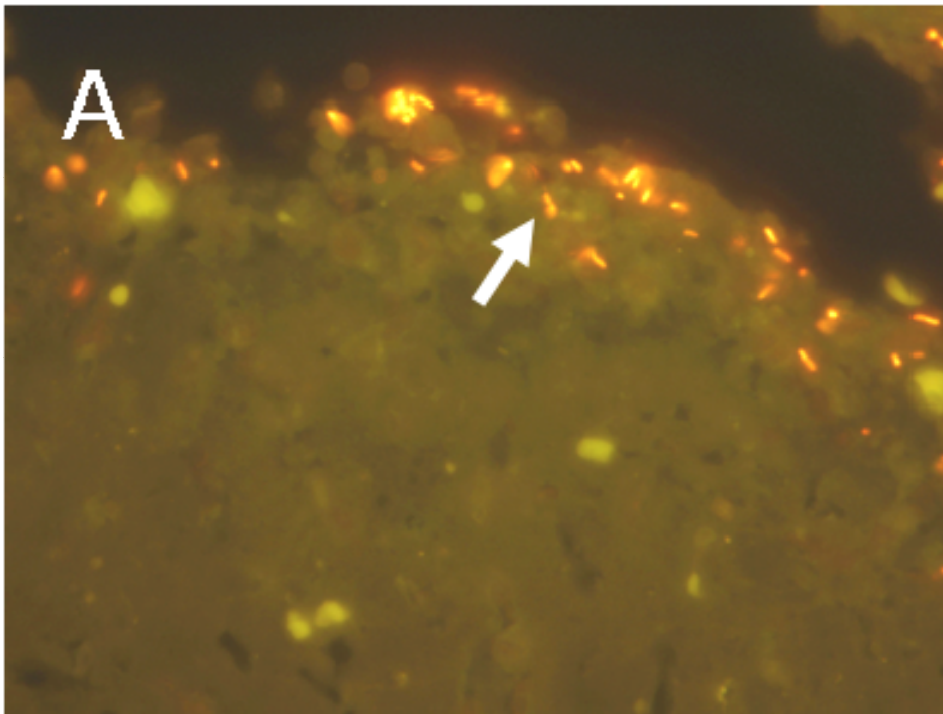
- Enters the acute wound within 6 hours
- Removes debris and bacteria by phagocytosis
- Attracts other immunological components
- Their function is gradually taken over by macrophages
- PMN normally disappears after 48 hours



# *Pseudomonas aeruginosa* in the Chronic Wound

A: Planktonic *P. aeruginosa* in a chronic wound

B: Microcolonies of *P. aeruginosa* in the same wound



# Chronic wounds with *Pseudomonas aeruginosa*

- Tends to be larger and heals slowly  
Gødsbøl et al, Int Wound J. 2006 Sep;3(3):225-31  
Madsen et al, APMIS. 1996 Dec;104(12):895-9.
- Have higher levels of metalloproteases, especially in combination with multiple bacteria

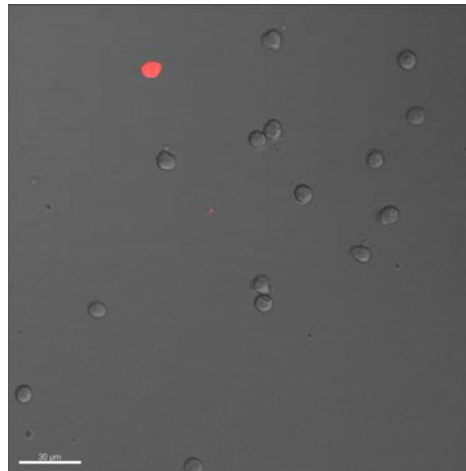
Lobmann R et al, Oral presentation DFSG 2005



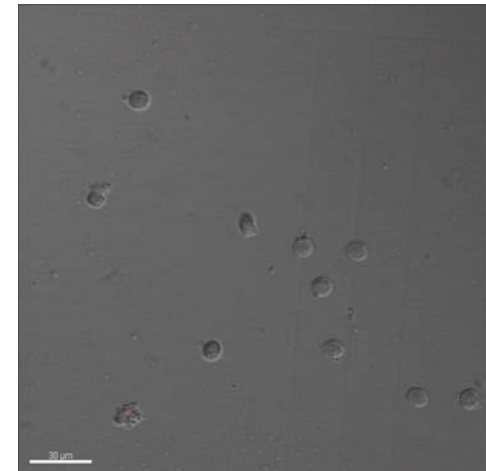
# *Pseudomonas* and PMN

- Quorum Sensing producing *P. aeruginosa* eliminates PMN
- Quorum Sensing blocked *P. aeruginosa* do not!

Sterile filtered supernatants from  
wt



QS mutant

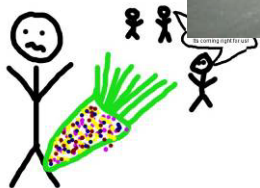




# Diabetic foot ulcer



# The story continues





# Still working on the same case

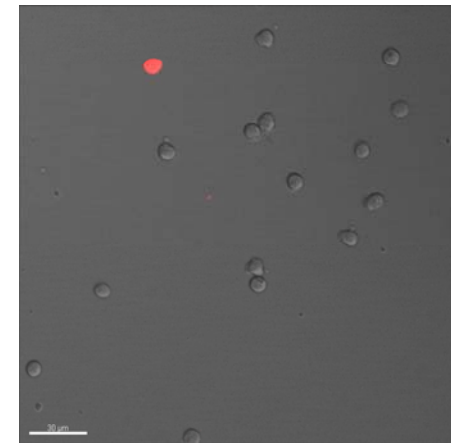


# Experimental infection with *Pseudomonas aeruginosa*

- Rabbit keratitis model
  - Elevated MMP-2 and MMP-9
  - Decreased TIMP-1 and TIMP-2

Ikema K et al, Exp Eye Res. 2006

Dec;83(6):1396-404. Epub 2006 Sep 11.



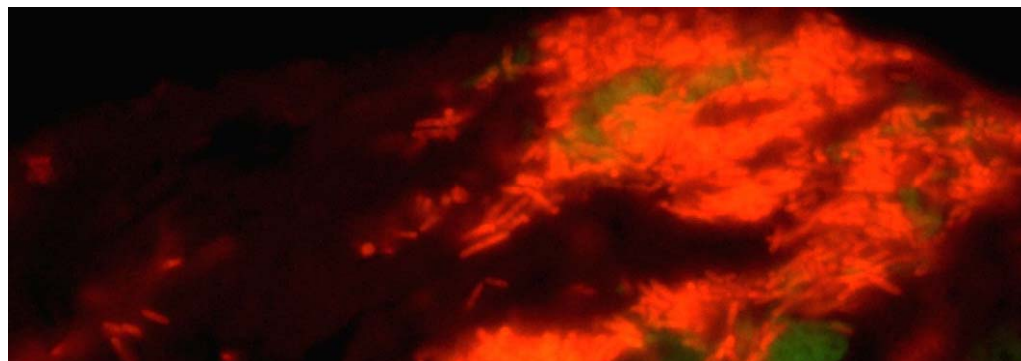
# *Pseudomonas aeruginosa* escapes elimination from the chronic wound

By

- Biofilm formation
  - Increases antibiotic tolerance
- PMN elimination
  - Quorum Sensing dependent virulence factor
    - Rhamnolipid




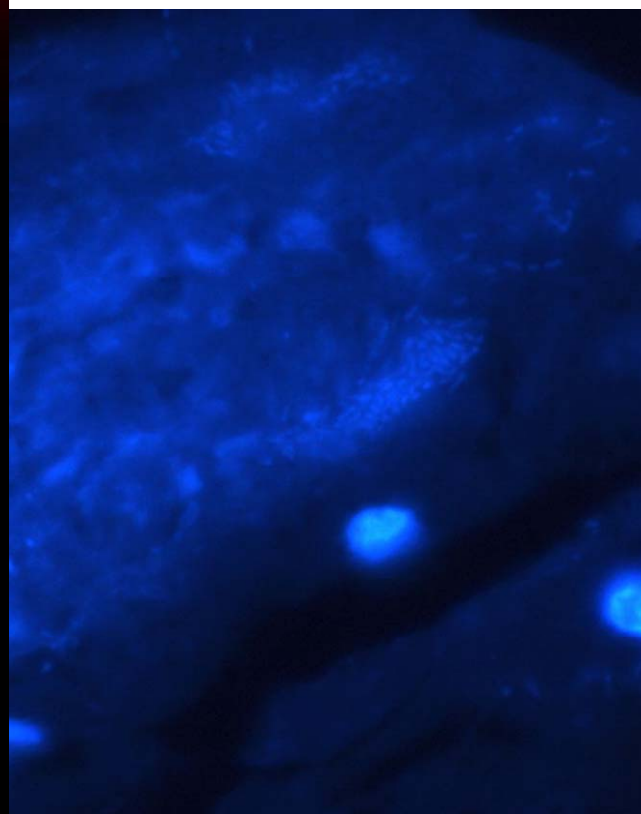
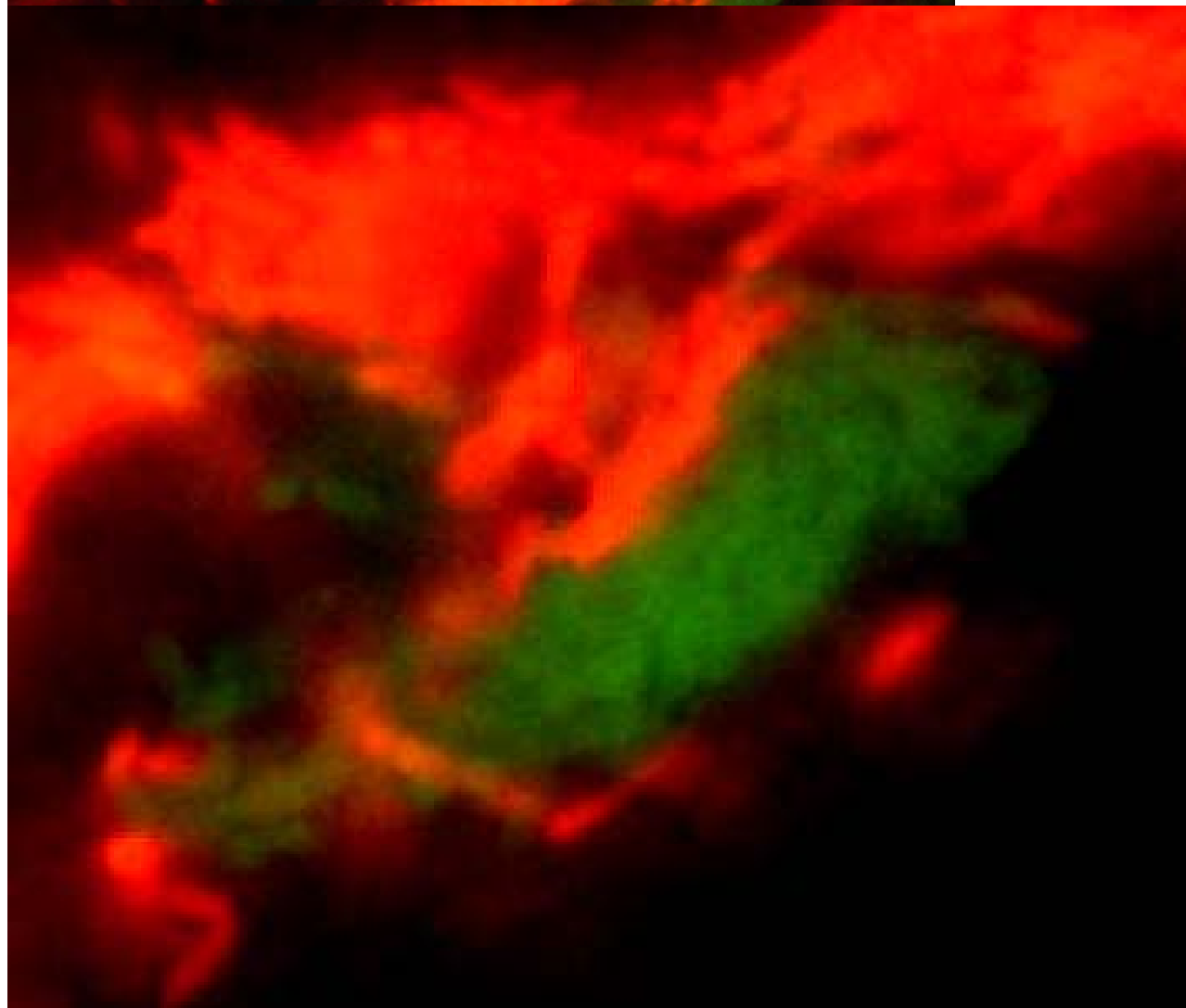




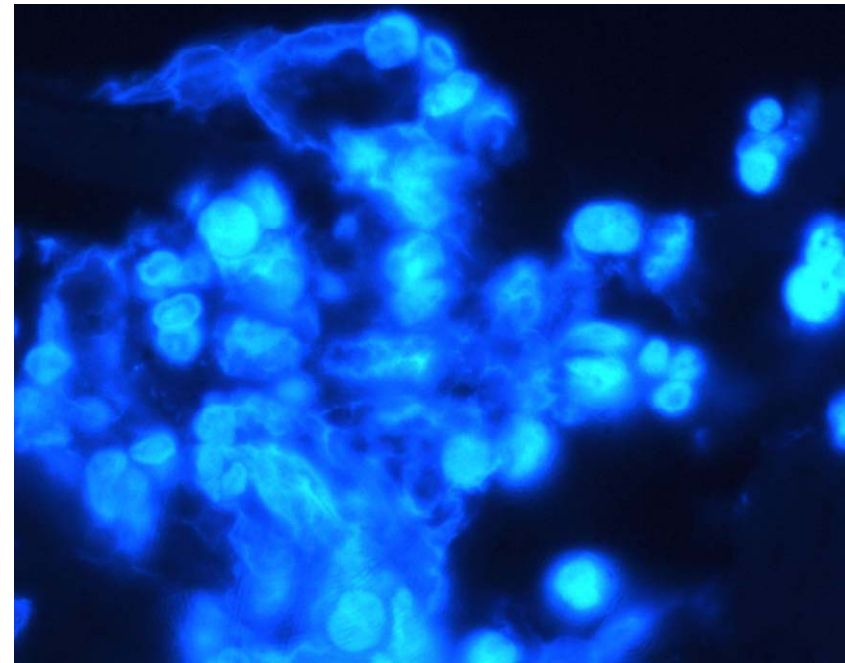
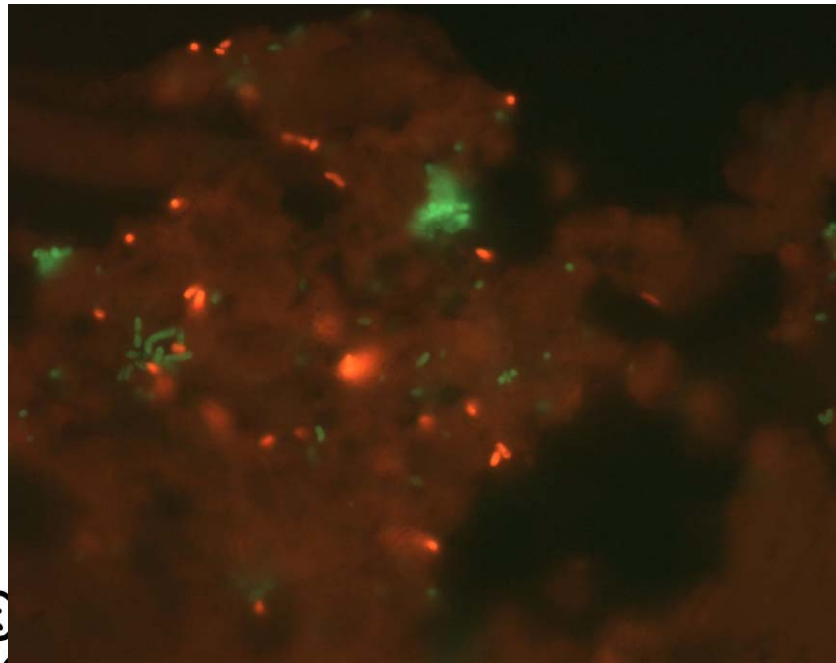
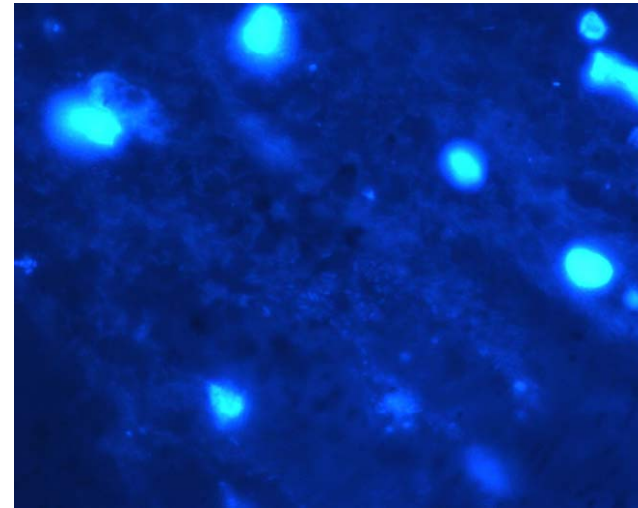
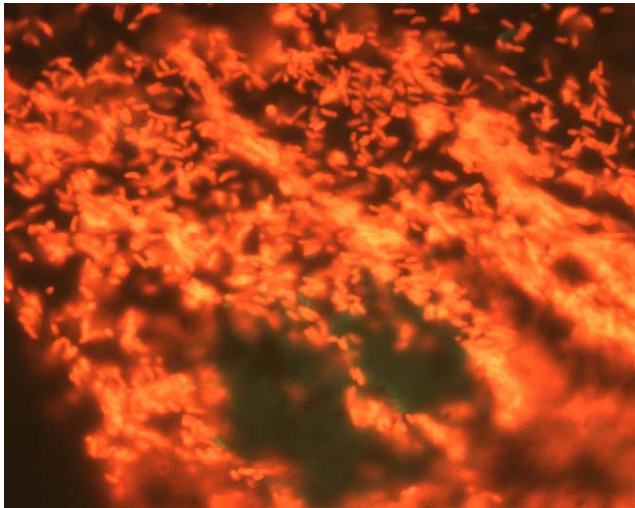
 PNA *P. aeruginosa*

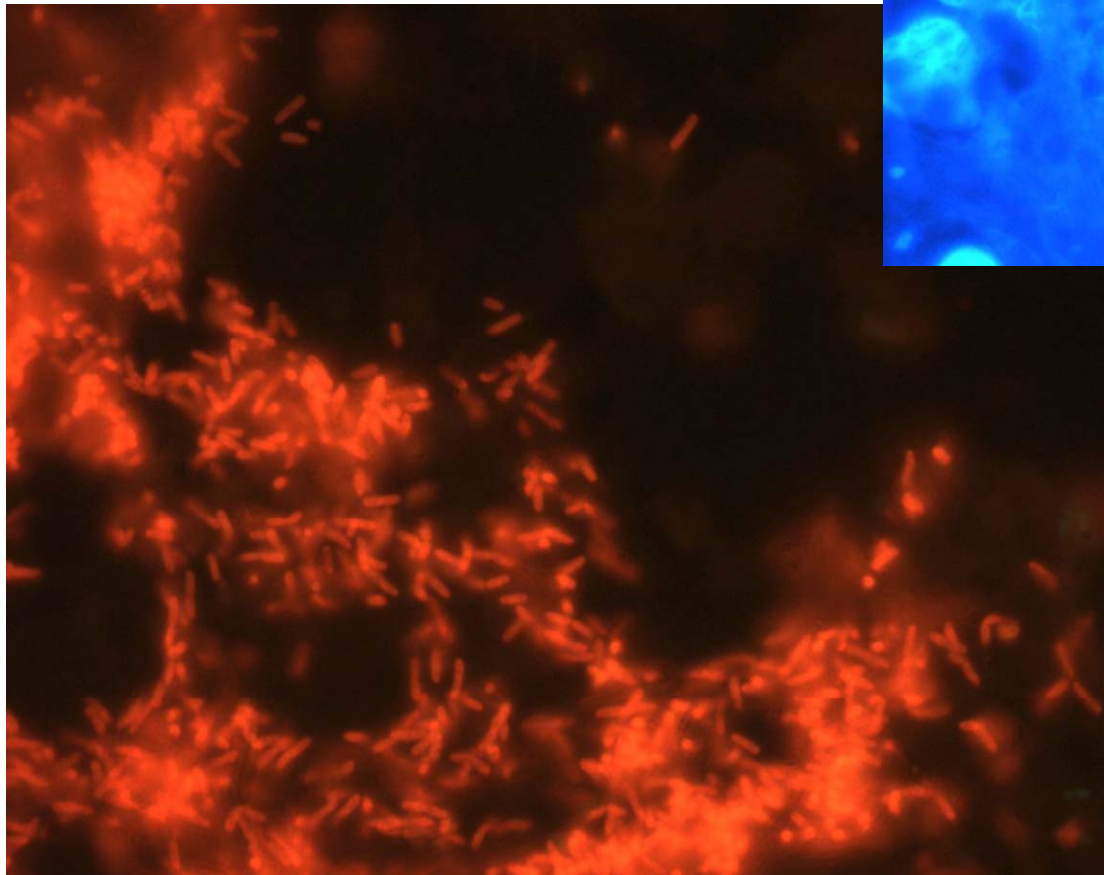
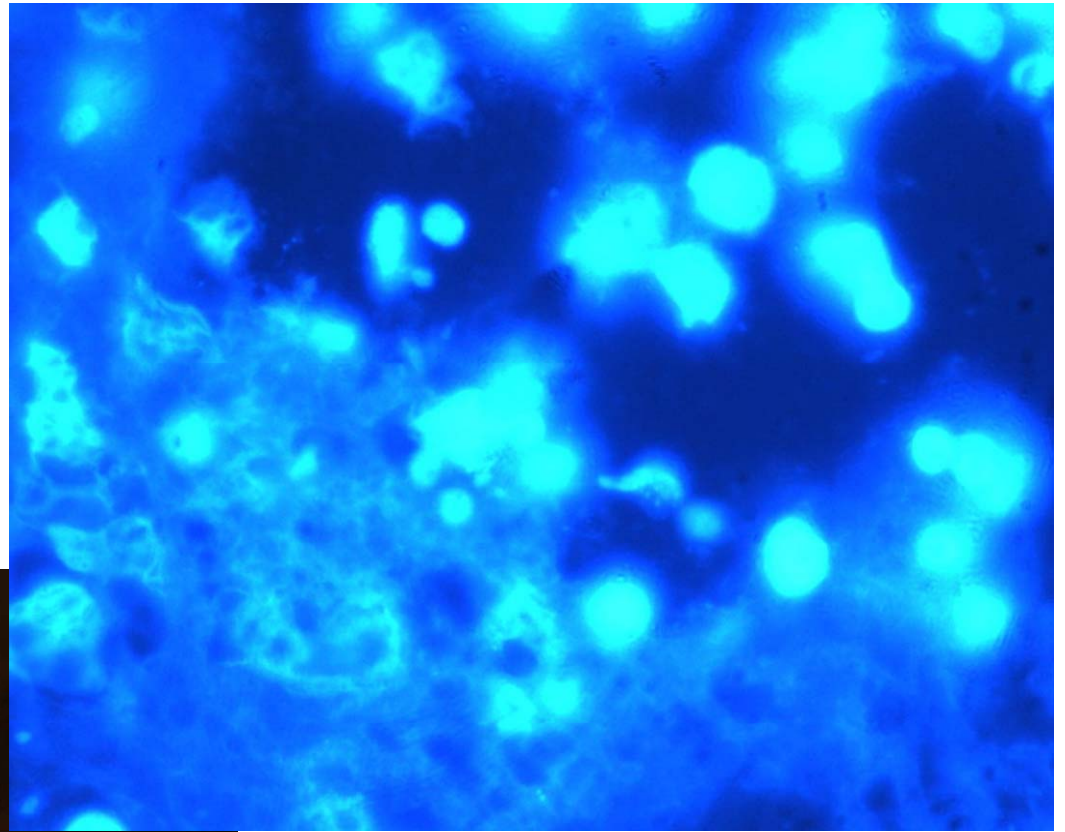
 PNA *Eubac*

 DAPI



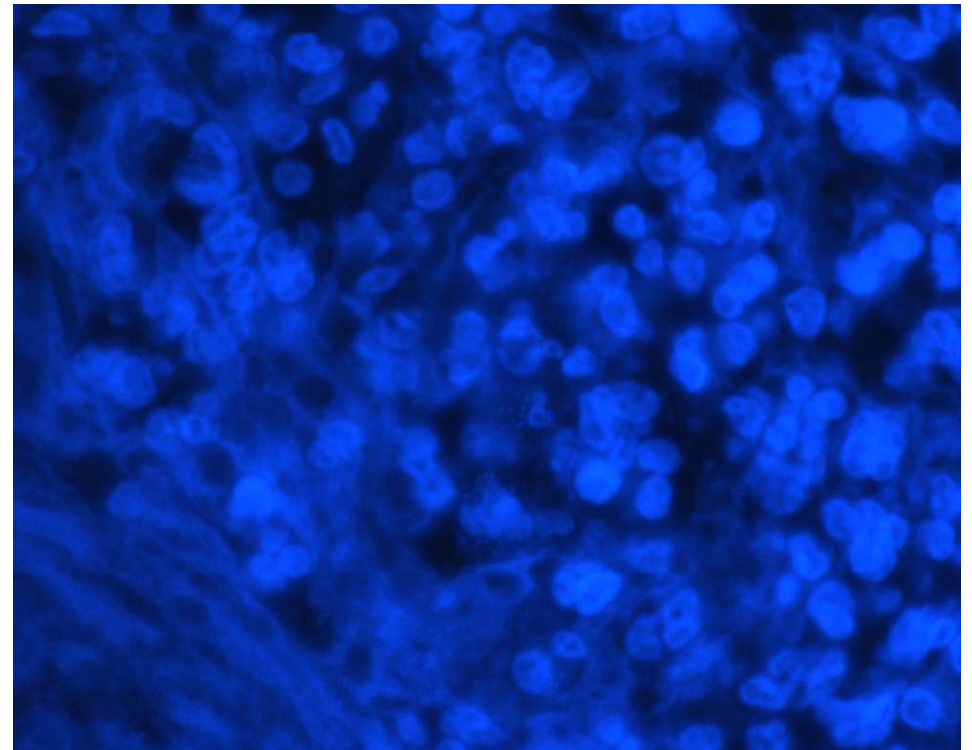
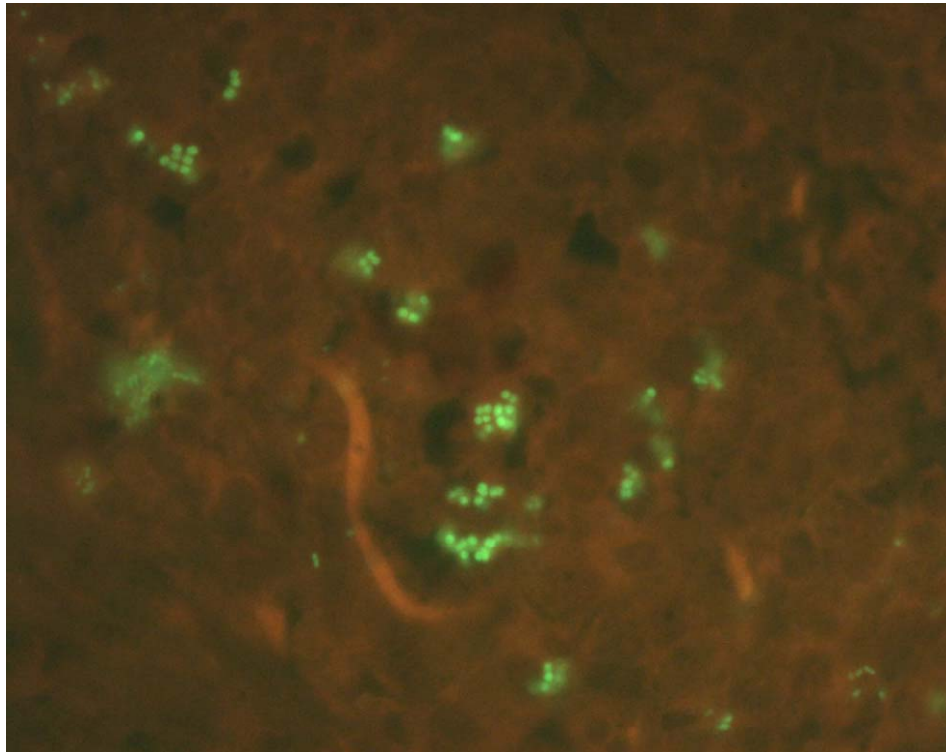
Patient 11: *Staph. aureus*, *Enterococcus faecalis*, *P. aeruginosa*, *Klebsiella pneumonia*



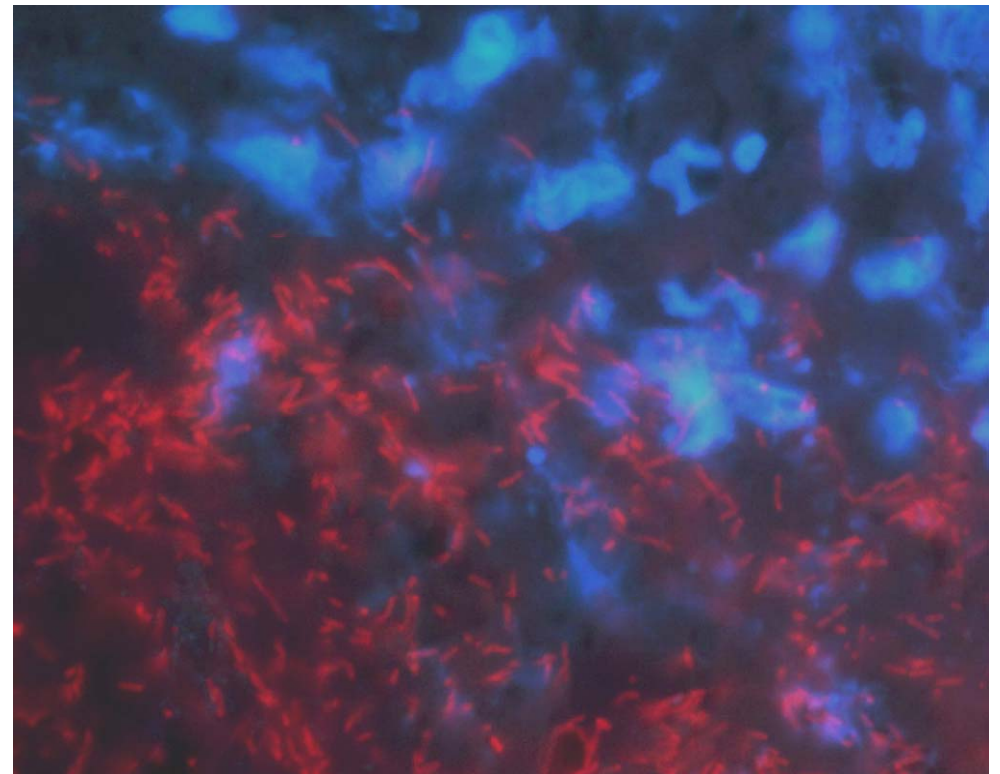
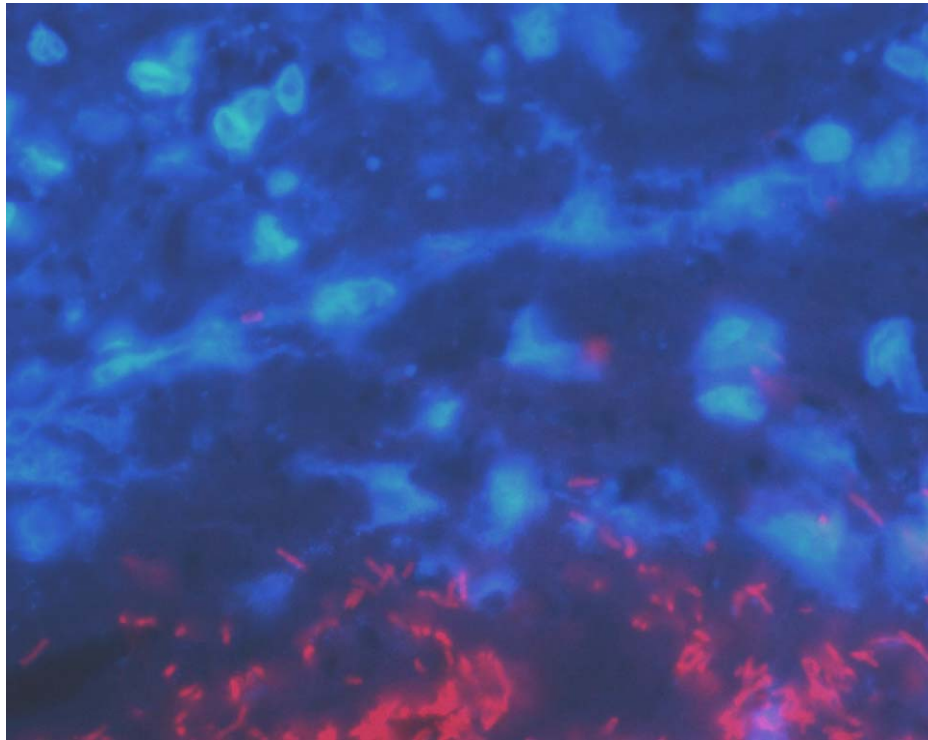




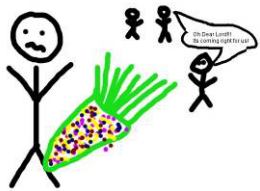
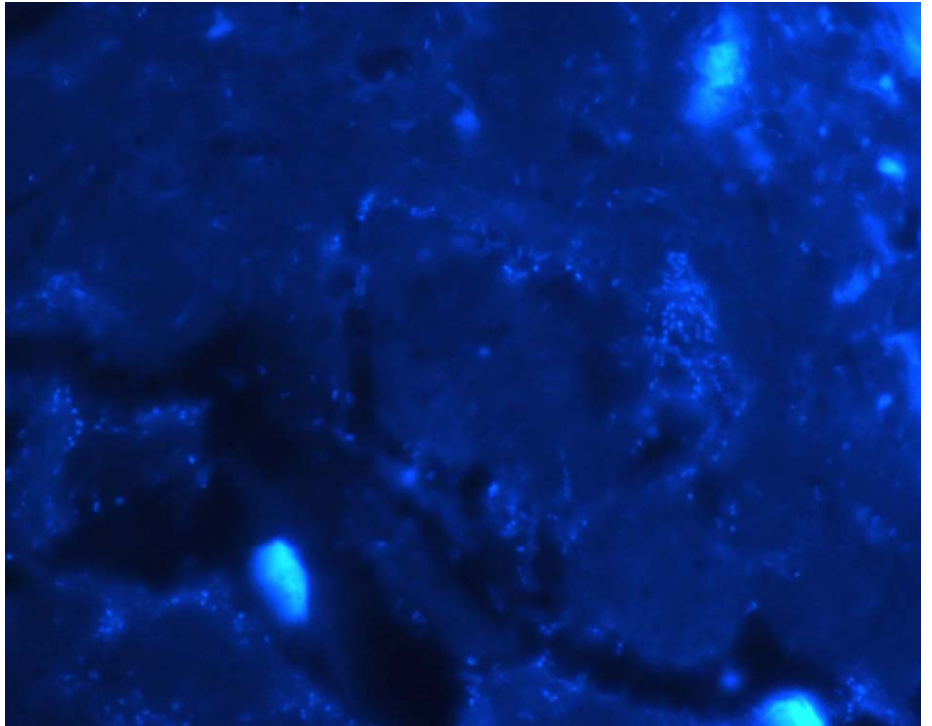
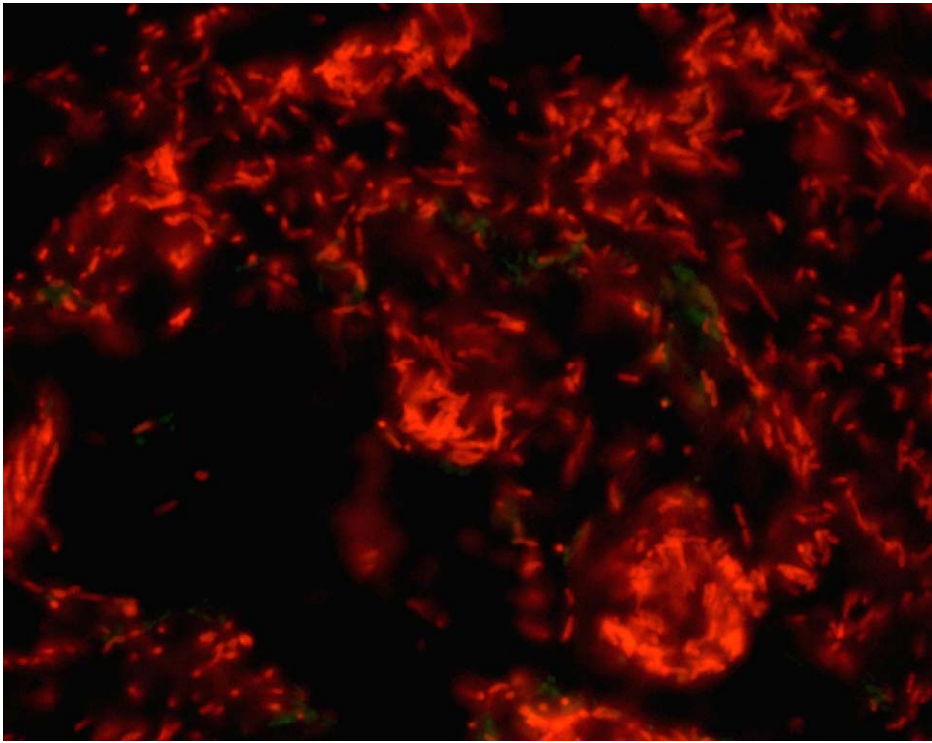
Patient 2: *Staph. aureus*, *Enterococcus faecalis*, *P. putida*, *Corynebacterium* species

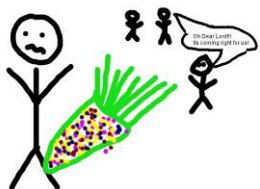
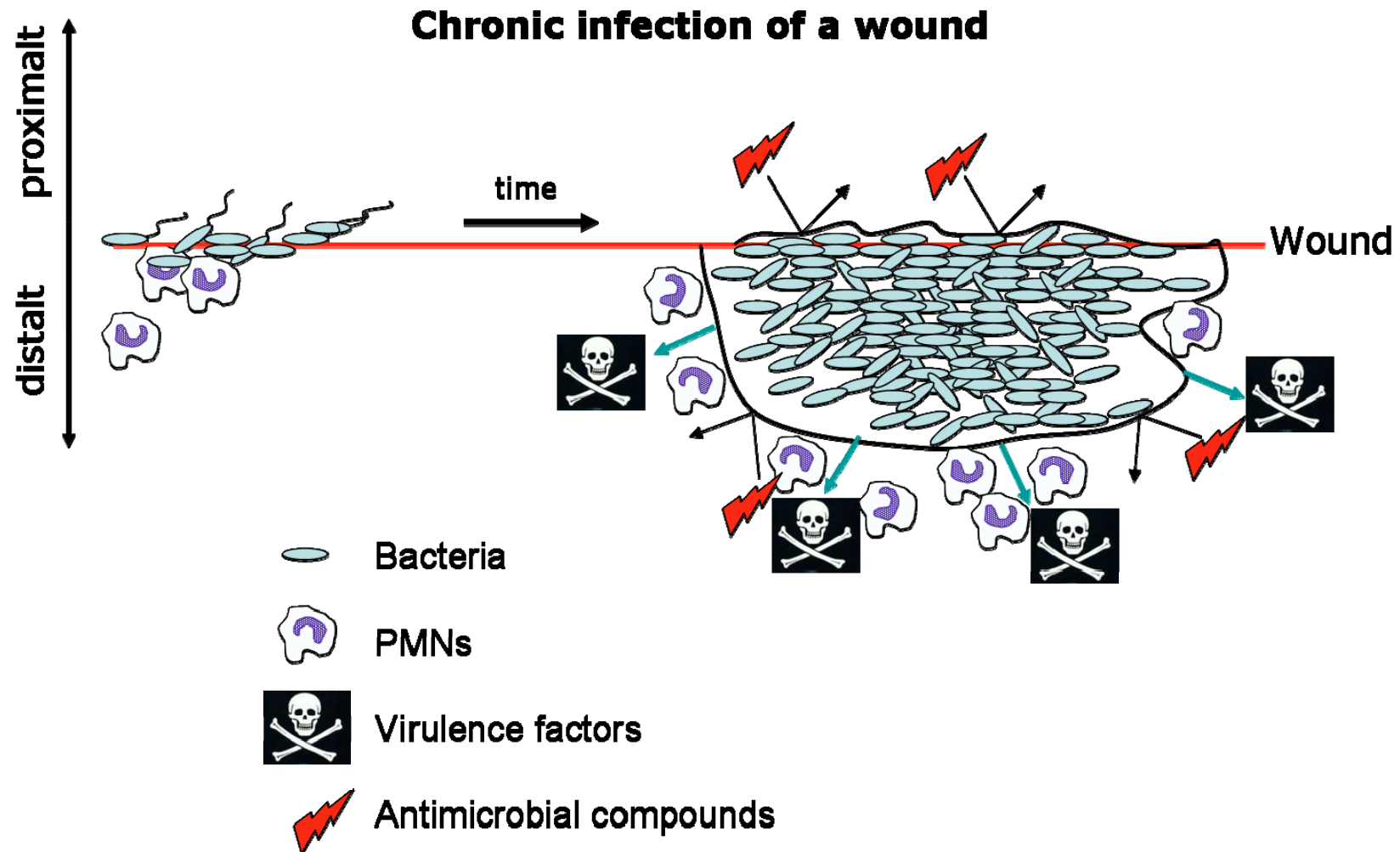


Patient 1: *P.A*









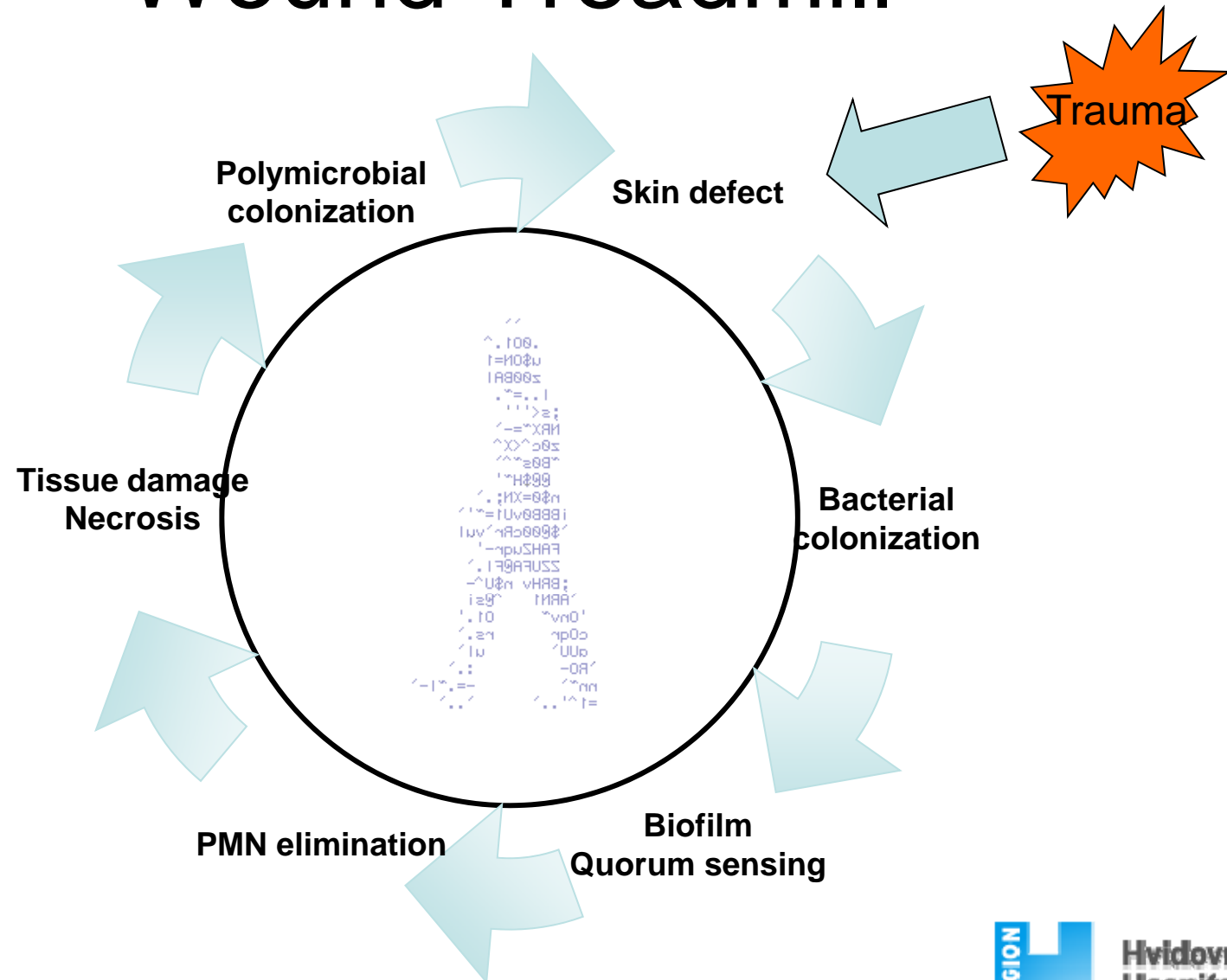
# In conclusion

We propose

- *Pseudomonas aeruginosa* escapes elimination by biofilm formation
- *Pseudomonas aeruginosa* deteriorates wound healing by PMN elimination
- Presence of *Pseudomonas aeruginosa* explains chronic wound characteristics
  - Elevated MMP
  - Decreased TIMP
  - Chronic inflammatory state (PMN influx)



# Wound Treadmill



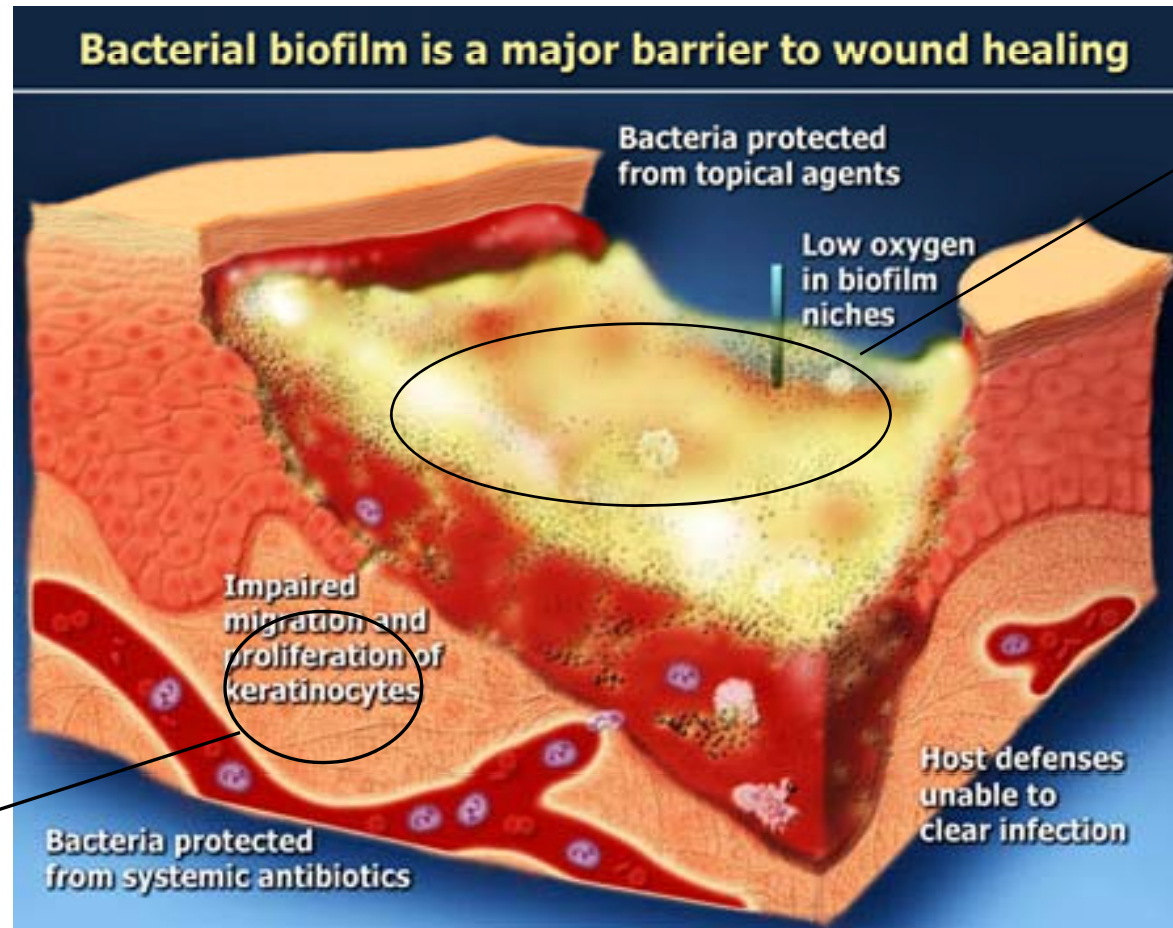
# Controversies

- Normal bacterial biofilm is associated with surfaces
  - Implants
  - Catheters
- This has led to the assumption that bacterial biofilm is on the surface of chronic wounds



# Biofilm in Chronic wounds?

Center for Biofilm Engineering, Montana



Here?

Here too?





# Procedure: Split Skin Transplant for Chronic Ulcer

- ***Excision:***
- ***Transplantation:***





# Results

	Intact
+ <i>P. aeruginosa</i>	33,3% (8/24) P=0,001
- <i>P. aeruginosa</i>	73,1% (49/67) P=0,001

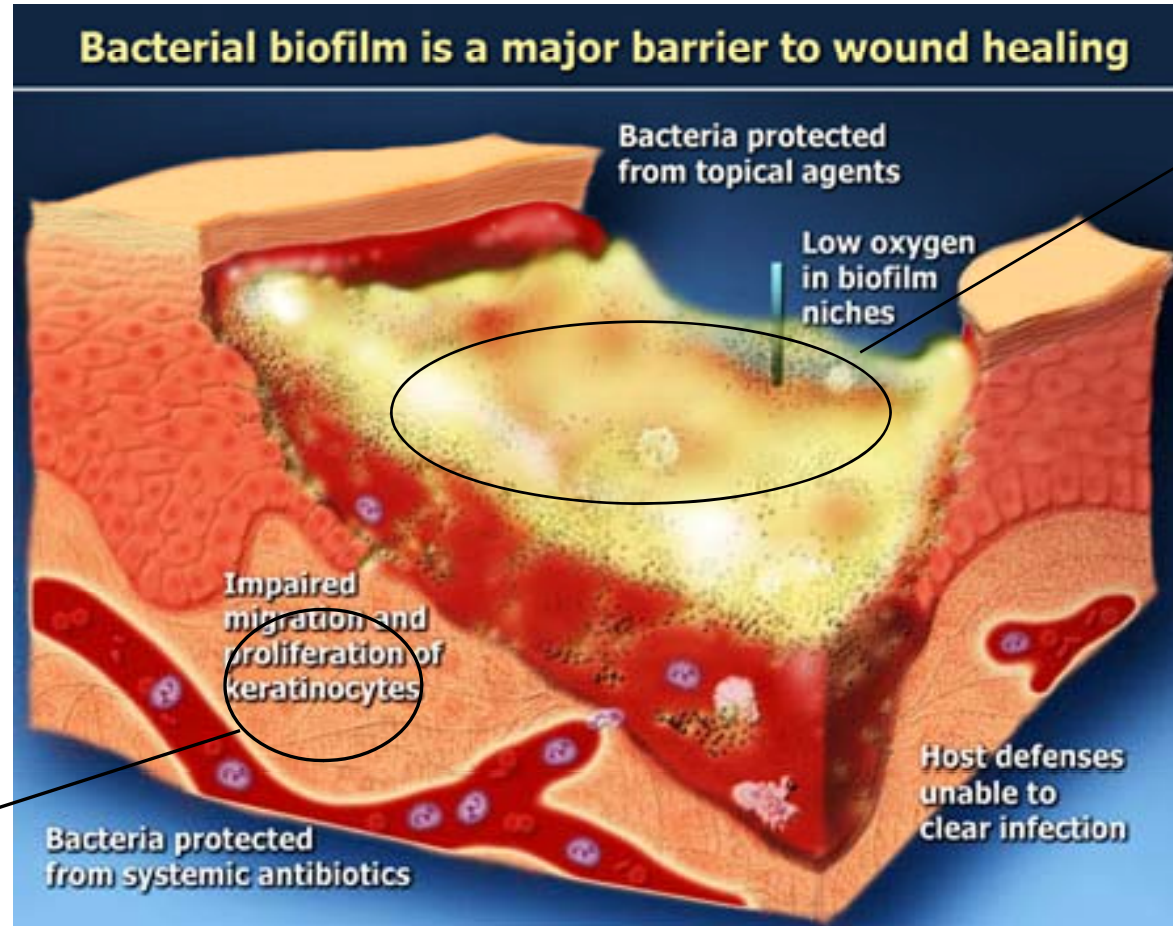
Success Rate of Split-Thickness Skin Grafting of Chronic Venous Leg Ulcers Depends on the Presence of *Pseudomonas aeruginosa*: A Retrospective Study

Høgsberg et al.  
(2011) *Plos One*



# Biofilm in Chronic Wounds?

Center for Biofilm Engineering, Montana

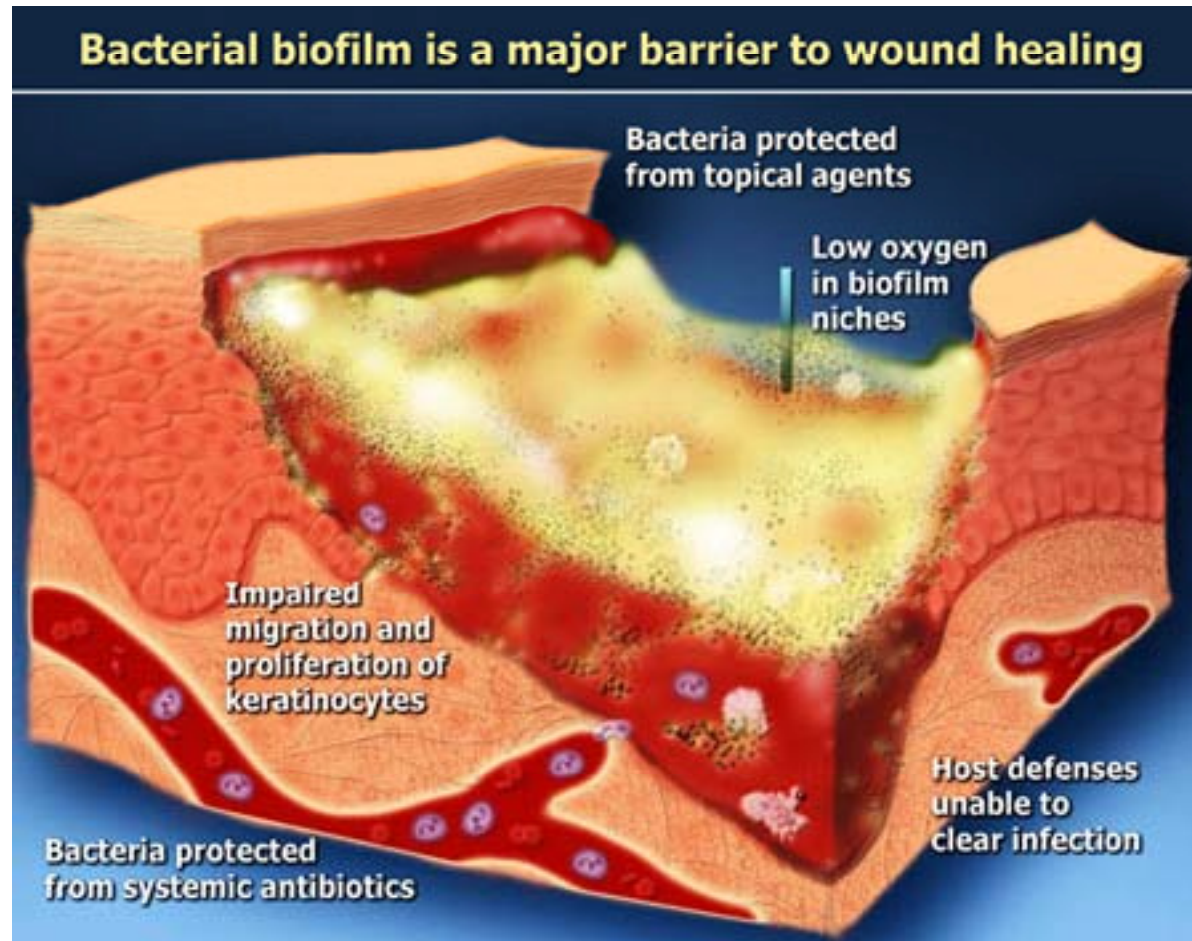


Here!

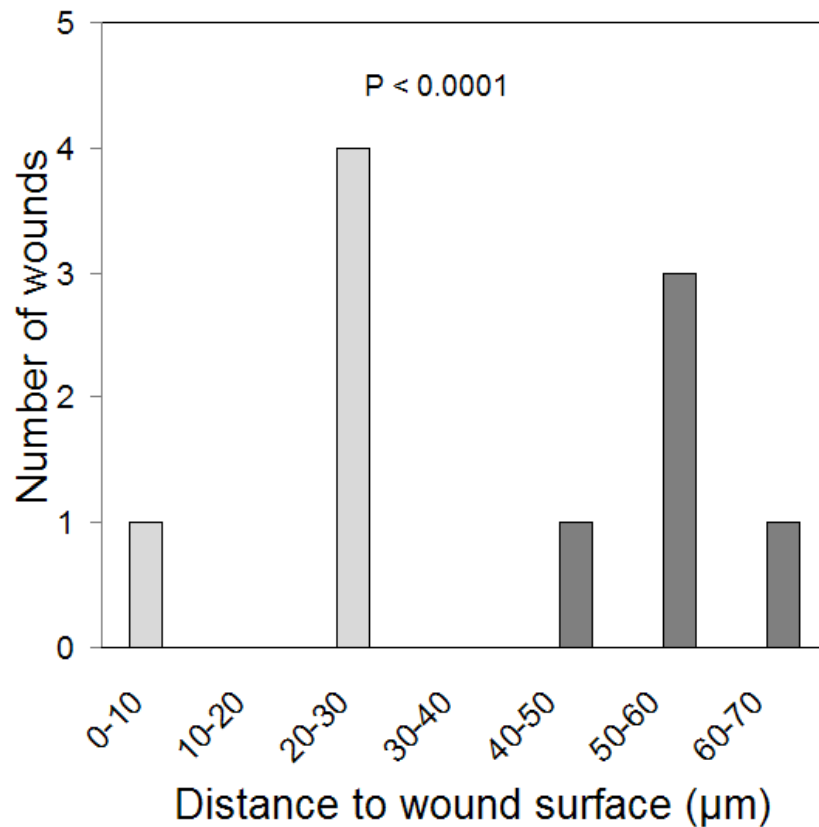
Here too!



# Swab or Biopsy?



# Distance from Surface

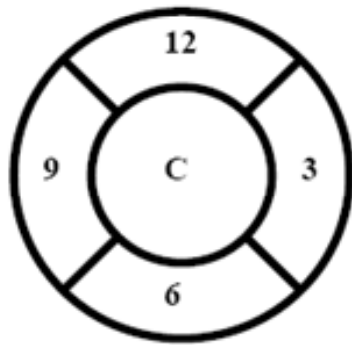


**Figure 2.** The distribution of the distances from the wound surface to the center of mass of *S. aureus* aggregates (□) or *P. aeruginosa* aggregates (■). The distances are average values obtained from analysis of 15 images for each wound sample.

Nonrandom Distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in Chronic Wounds  
Mustafa Fazli et al. JCM dec. 2009

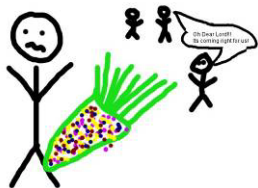


# Heterogeneity



		<i>S. aureus</i>		<i>P. aeruginosa</i>	
method		Wound E	Wound F	Wound E	Wound F
C	q-PCR	<b>89±11%</b>	<b>200±13%</b>	<b>510±18%</b>	<b>920±9%</b>
	cultivation			+	
3	DGGE	+	+		
	q-PCR	No sample	<b>86±8%</b>	No sample	<b>300±13%</b>
	cultivation				
	DGGE		+		
6	q-PCR	<b>240±10%</b>	<b>290±8%</b>	<b>760±7%</b>	<b>8200±8%</b>
	cultivation			+	
	DGGE	+	+		
9	q-PCR	<b>310±13%</b>	<b>80±5%</b>	<b>47±9%</b>	<b>800±10%</b>
	cultivation			+	
	DGGE	+	+		
12	q-PCR	<b>180±8%</b>	<b>93±12%</b>	<b>280±3%</b>	<b>15±5%</b>
	cultivation			+	
	DGGE	+	+		

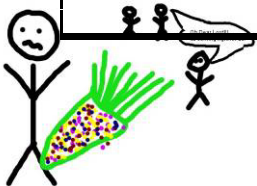
Influence of Microorganisms on the Healing of Skin Grafts from Chronic Venous Leg Wounds  
Wulf et al. InTetch Book chapter



# Sampling Technique

## Pro's et Con's

	Swab	Biopsy
Pro	Large area Non-invasive	More than surface Larger sample size
Con	Only surface	Small area Invasive





# A Shift of Paradigm?

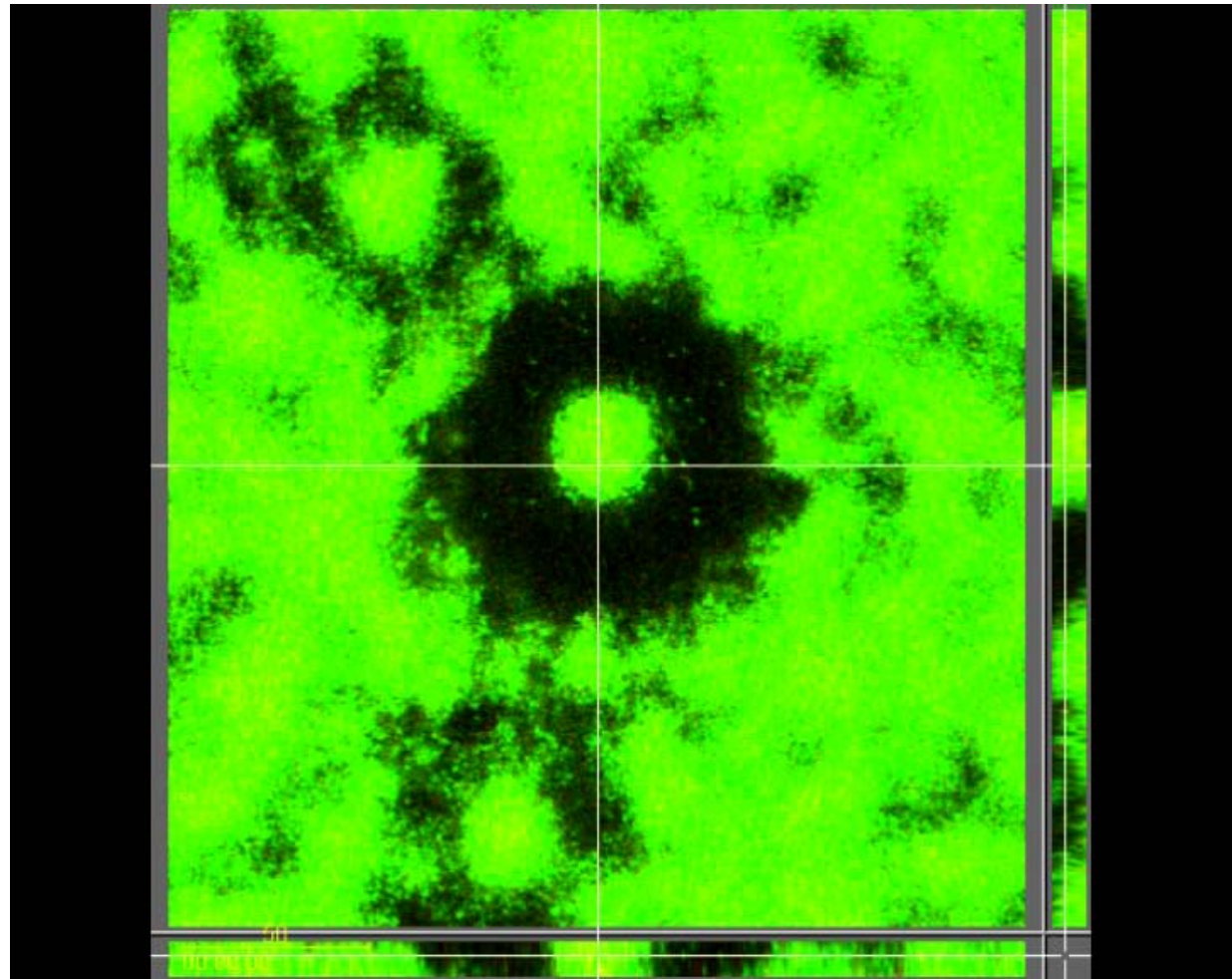
- Antibiotics and surgery is often not enough to eradicate bacterial biofilm
- We are looking for a substance that readily kills bacteria throughout the biofilm and is tolerated by the tissue
- This substance could be buffered Acetic acid!
  - 0,5% acetic acid eradicates *P. aeruginosa* (gram negative)
  - 1,0 % acetic acid eradicates *S. aureus* ( gram positive)
  - pH dependant effect



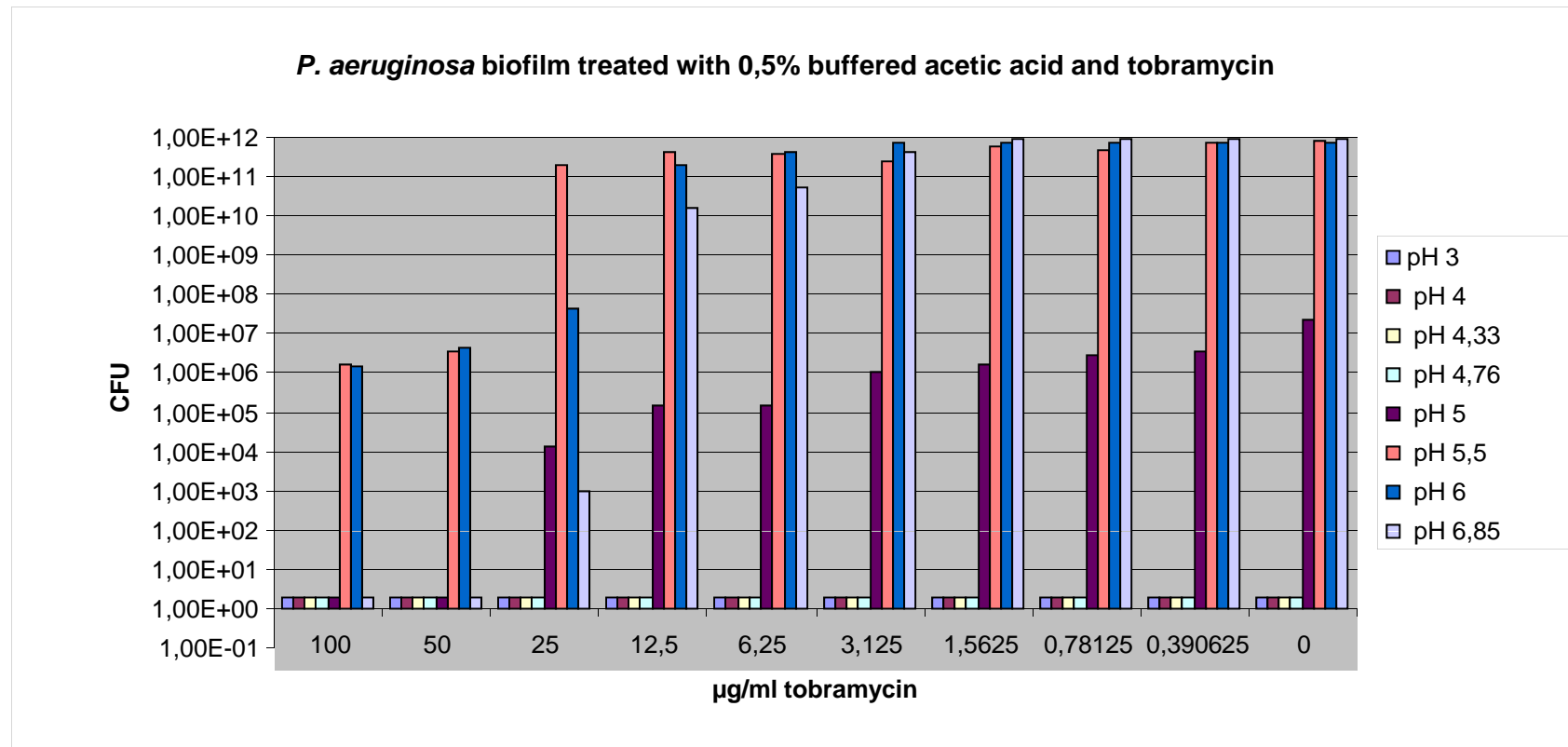
# How did this get started?

Acetic acid is used to treat middle ear infections at Rigshospitalet, Copenhagen

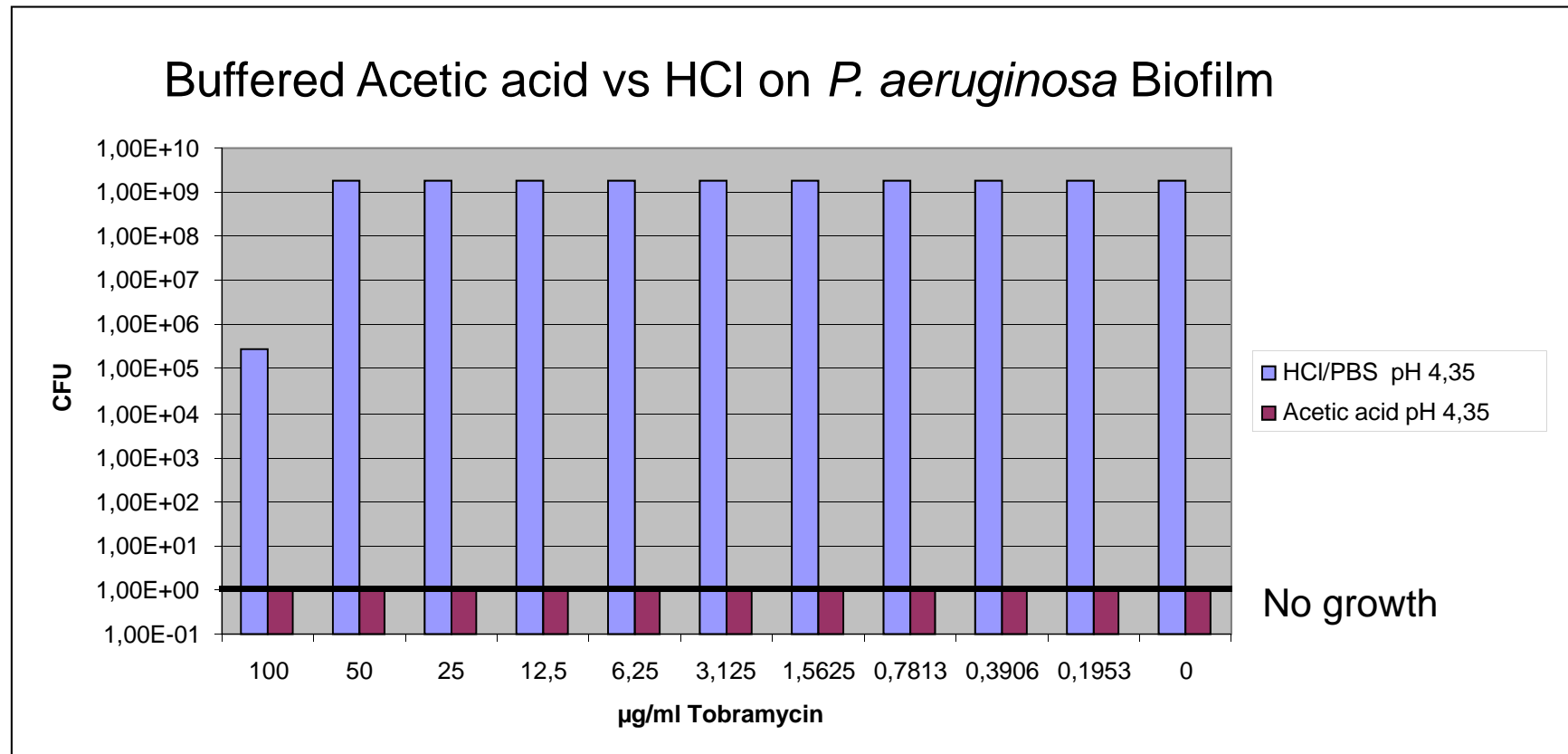
Initial experiments were too good to be true....



# *In Vitro* – pH dependency



# *In Vitro* - HCl vs. Acetic Acid



# Case 1: Patient with recent heel ulcer

## Anamnesis

- A 38-year old male with T2DM associated neuropathy was presented to Copenhagen Wound Healing Clinic. A heel ulcer was obtained during a vacation due to strenuous walking.

Prior history of treatment with no apparent improvement in wound healing (over period of three months)

- Off-loading, therapeutic shoes, and Aircast.
- Wound treatment with silver dressings and compression.
- Several courses of antibiotics.

Treatment of wound with phosphate buffered acetic acid (patient continued antibiotic therapy)

- 6x20 minutes per day, for 10 days (continuous) in combination with NPWT Therapy



# Case 1: Patient with recent heel ulcer

Day 0



Day 36 (split skin graft 10 days prior)





# Case 2: Patient with chronic foot ulcer

## Anamnesis

- A 54-year old woman with a past history of IV-drug abuse. Difficult to treat venous insufficiency and a long history of chronic leg ulcers. The wound is infected with *P. aeruginosa* and *E. faecalis*. Have been treated for the last 8 years at Copenhagen Wound Healing Clinic.

## Prior history of treatment with recurrent leg ulcers

- Compression and various dressings .
- Numerous antibiotic treatments.
- Numerous skin transplants which have been lost.

Treatment of wound with phosphate buffered acetic acid (patient continued antibiotic therapy with meropenem) 6x20 minutes per day, for 10 days (continuous) in combination with NPWT Therapy



# Case 2: Patient with chronic foot ulcer

# Day 0



## Day 379



# Intellectual Property Rights

- A PCT patent has been filed covering the active substance “WO2009/155931 A1”
- Wound dressings incorporating the active substance is also claimed



# Let Us Return to the Paradox

- Most diabetic foot ulcers or other chronic wounds heals with prober off-loading, treatment of infection, edema reduction and management of ischemia
- How can this paradox be explained?



# Healing Despite of Biofilm

- Not all biofilms are harmful
- Virulent biofilms can change virulence pattern
  - Change of habitat due to
    - Off-loading
    - Edema reduction
    - Infection control
    - Alterations in nutrition factors
    - No apparent reason





# What Needs to be Done in Research

- How to identify the wounds that are non-healing due to biofilm
- How do we measure virulence?
- Does the actual wound contain harmful biofilm?
- How do we conquer biofilms?
  - Jamming the communication
  - Blocking virulence factors
  - Electric field therapy
  - New drugs that kills bacteria throughout the biofilm



# Until then ...

## International Consensus on the Diabetic Foot

by the International Working Group on the Diabetic Foot

Opinion Piece

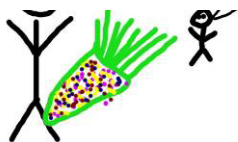
### The fight against biofilm infections: Do we have the knowledge and means?

When a ship arrives on the shores of an unknown territory with scarce or no information of what is beyond the horizon, it is only confidence in the capacity and the skills of the crew and hardware that will convince the commander that the land can be taken. Intelligence is of outmost importance. Do we have the intelligence in the battle against biofilm infections to win?

In the present paper we will list what we believe is the key knowledge today and identify what science lacks, in order to suggest research strategies to resolve biofilm infections.

#### A Paradox:

*How wonderful that we have met with a paradox. Now we have some hope of making progress.* Niels Bohr (1882-1962)



#### Communication and virulence factors

Communication between bacteria is pointed out to be a target for intervention. Quorum Sensing (QS) in general and between *Pseudomonas aeruginosa* specifically is only a fragment of the communication between the bacteria. The N-acyl homoserine lactone QS signal molecule in *P. aeruginosa* will trigger the production of virulence factors such as rhamnolipids that, in vitro, can eliminate Neutrophils<sup>1</sup>. Blocking or modification of QS, in theory, will enable the immune system to eradicate the bacteria even in mature biofilms. However the QS molecules differ between Gram-positive and Gram-negative bacteria and even within these. A single drug to regulate all the harmful effects of QS is hardly imaginable.

We have only a little overview of the communication in multi-species biofilm and of the communication between different mono-species



<sup>1</sup>Klaus Kirketerp-Møller  
MD



<sup>2,3</sup>Thomas Bjørnsholt,  
PhD



- Follow the guidelines and..
- The residual ulcers are likely to contain harmful biofilm
- Please refer to EWMA Journal May 2011 for more details

# Take Home Message

- All chronic wounds contain biofilm
- Not all chronic wounds stay chronic due to biofilm
- Biofilm is not only present on the surface
- Today we do not have the tools to identify harmful biofilm



# Acknowledgements

Michael Givskov  
Thomas Bjarnsholt  
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Bo Jørgensen  
Copenhagen Wound Healing  
Center  
Anders Andersen  
Statens Serum Institut  
Danish National Hospital  
Rigshospitalet

AdvanDx





1<sup>ST</sup> ANNOUNCEMENT – CALL FOR ABSTRACTS



30th Annual meeting of the European  
Bone and Joint Infection Society



**EBJIS 2011**

Biofilm and Health Economics  
in Bone and Joint Infections



15-17 September 2011  
The Panum Institute,  
Copenhagen, Denmark

[www.ebjis2011.org](http://www.ebjis2011.org)

Thank you for your  
attention

